Video Article

Semi-automated Optical Heartbeat Analysis of Small Hearts

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Abstract

We have developed a method for analyzing high speed optical recordings from *Drosophila*, zebrafish and embryonic mouse hearts (Fink, et. al., 2009). Our Semi-automatic Optical Heartbeat Analysis (SOHA) uses a novel movement detection algorithm that is able to detect cardiac movements associated with individual contractile and relaxation events. The program provides a host of physiologically relevant readouts including systolic and diastolic intervals, heart rate, as well as qualitative and quantitative measures of heartbeat arrhythmicity. The program also calculates heart diameter measurements during both diastole and systole from which fractional shortening and fractional area changes are calculated. Output is provided as a digital file compatible with most spreadsheet programs. Measurements are made for every heartbeat in a record increasing the statistical power of the output. We demonstrate each of the steps where user input is required and show the application of our methodology to the analysis of heart function in all three genetically tractable heart models.

Protocol

Click here to view an overview of the semi automated heartbeat analysis (SOHA) procedure.

Semi-automated optical heartbeat analysis

1. Preprocess -

This step provides information on the diastolic and systolic heart diameters.

Heart edges are identified and marked during maximum diastole and systole. Optical recordings can be advanced at slow speeds and even one frame at a time allowing precise identification of the frames where maximal contraction and relaxation of the heart occur. One pair of marks identifying the diastolic edges and one pair identifying the systolic edges are made. Marks can also be made in duplicate at different horizontal locations along the heart.

The heart diameters obtained in this step are also used to calculate an additional measurement that provides an estimate of the contractility of the heart. This measure is the percent fractional shortening (%FS) and it represents the extent to which the heart edges move toward each other during a contraction. The formula used to calculate %FS is:

(((Diastolic diameter - Systolic diameter)/Diastolic diameter) x 100)

Note - The points identified in this set are used only for diameter and fractional shortening measurements and are not used for movement detection.

Example: In Drosophila, point mutations in myosin heavy chain's transducer domain, a region known to modulate the motor's enzymatic activity, result in hearts that are dilated or restricted depending upon whether they suppress or enhance myosin's ATPase activity (Cammarato, et al. 2008). These types of measurements cannot be made in intact flies as it is usually not possible to identify heart edges along the entire length of the heart tube through the cuticle and the fat, (compare Wildtype, D45 and Mhc5 M-modes in "M-mode Figure" below) which normally surround the heart. Measurements made when visualizing the heart in intact flies with other commonly employed methods are usually restricted to the most anterior portion of the heart, the conical chamber region. It is also not possible to obtain this dynamic measurement in fixed tissue.

2. Movement Detection-

This step is done automatically by the program and uses two different algorithms to analyze movement in each movie. Briefly, the first algorithm, the "Average Frame Darkness" algorithm uses a frame by frame approach. This algorithm averages the darkness intensity for an entire movie frame, normalizes the value to an interval between 0 and 1, and plots the normalized average for every frame in the movie over time. The second approach, the "Pixel by Pixel" algorithm, analyzes the change in darkness of all the individual pixels from one frame to the next. Pixels that exhibit intensity changes above a preset threshold are summed for each frame and plotted for every frame in the movie over time.

3. Check Intervals-

The output from the two movement detection algorithms is displayed in this module with the "Average Darkness" output displayed in a window above the "Pixel by pixel" output window. In order to be sure that the detection intervals agree with movement in the actual movie we display an edge-trace or M-mode made from the frames being analyzed directly below the algorithm output windows. The M-mode is made by digitally cutting slices one pixel wide through the heart from each frame in the movie and aligning them horizontally to provide a snapshot of the heart

edge movements over time. In the "Pixel by pixel" window the identified diastolic intervals are indicated by a horizontal green line with the number of frames contributing to that interval directly above. Systolic intervals are identified as the interval between two successive diastoles. The beginning of a systole is indicated in the M-mode by a vertical blue line and the end is indicated by a vertical red line. With this display it is relatively easy to see if the algorithm output agrees with the actual heart movements.

Although movement detection is performed automatically the output must be checked for accuracy. There are several reasons for this; one is that there is often an underlying, slow wave fluctuation in light levels not related to the heart movement. This can usually be eliminated by use of a built in high pass filter. A second reason has to do with the sensitivity of the "Pixel by pixel" approach. The output from this algorithm is typically two movement traces, one for the contraction movement and one for the relaxation movement. In older flies and in some mutants the length of time between these two movements becomes relatively long and the program tries to interpret the two movements as two separate beats. This can be corrected by asking the program to compare the output of the Pixel by Pixel algorithm with that of the Average Frame Darkness algorithm, by clicking on the "Use darkness" box.

Example (refer to Video): In 5 to 7 week old flies the systolic intervals are typically much longer than in young flies. These long intervals are initially detected as two separate contractions separated by a short diastole. This can be corrected by selecting the "Use darkness" check box. In this case the program will compare the diastolic interval identified by the Pixel by Pixel algorithm with the Average Frame Darkness output. If the Average Frame Darkness output has a positive value, then any diastolic interval identified in the Pixel by Pixel algorithm will be ignored and the intervals will be correctly specified. The entire movie can be scanned in 10 second segments and accepted (click "Data OK") or rejected (click "Discard Data"). The Hi and Lo pass filter sensitivities can be manually adjusted to help more accurately define contraction intervals when employing the Darkness algorithm.

4. Output Statistics-

Once the output for a given movie has been accepted the program will use that information to automatically calculate a number of parameters. These include diastolic and systolic interval, heart period and heart rate, direction and velocity of the contraction wave, heart diameters, and %FS. In addition, the program has multiple methods for quantifying arrhythmicity reflecting non-uniform contraction and relaxation cycles (see Fink et al, 2009). Statistical output is provided for each movie individually and for the entire data set in the form of a comma separated value (.csv) file which can be opened in spreadsheet programs such as Microsoft Excel.

Table 1-

	Info from filename			Mean and median and standard deviation:												
File Name	Geno Type	Geno Group	Age	Heart Rate			Heart Period			Diastolic Interval			Systolic Interval			norm HP SD
old fly	REST	1.00	0.00	0.66	0.39	0.92	2.58	2.55	1.30	1.80	1.80	1.16	0.78	0.74	0.31	0.51
young fly	REST	1.00	0.00	2.10	2.11	0.03	0.48	0.48	0.01	0.21	0.21	0.01	0.26	0.26	0.01	0.01

	Diameters (in microns):					
File Name	1st Diastole	1st Systole	2nd Diastole	2nd Systole	Distance	Frac Shortening
old fly	54.67	32.80	54.67	32.80	56.23	0.40
young fly	99.97	56.23	95.28	46.86	66.78	0.47

Estimate of Arrhythmicity:						Contraction Velocity [um/s]:					
Nr large DI	Quota DI	Nr outlier DI	Nr large SI	Quota SI	Nr outlier SI	To Left: Nr	median	Nr dilation	To Right: Nr	median	Nr dilation
1.00	0.03	17.00	0.00	0.01	16.00	18.00	-32948.22	3.00	5.00	15026.11	0.00
4.00	0.00	0.00	0.00	0.00	0.00	62.00	-4553.52	0.00	0.00	NaN	0.00

An example of the output generated by the Semi-automated Optical Heart Analysis program.

5. Update Histograms-

This portion of the program displays all of the data on heart intervals for a dataset in histogram format. Since heart rates are variable we normalize the histogram output data. However, because the distribution of the intervals is not symmetric around a mean we use the median interval value for our normalization.

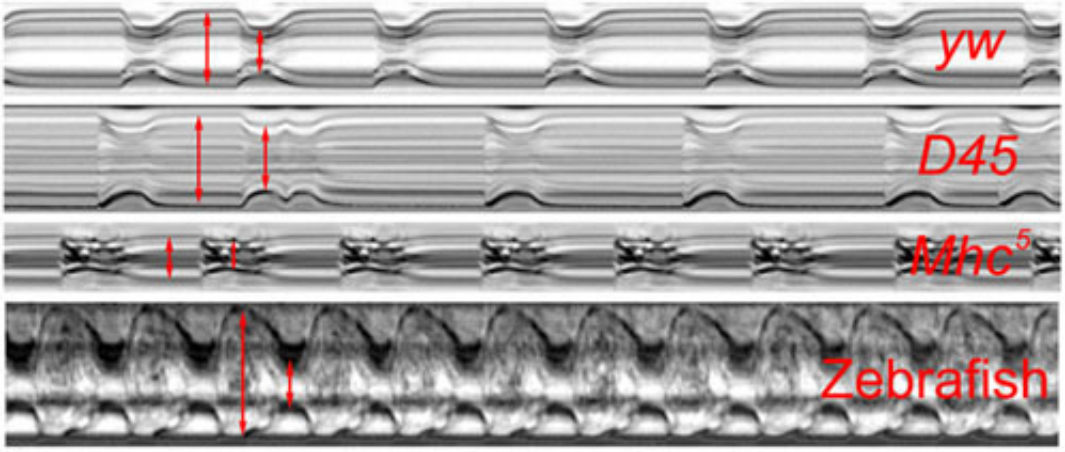
Example (refer to Video): Flies that are mutant for the KCNQ potassium channel show extremely irregular diastolic and systolic intervals. Typically the systolic intervals are much longer and more irregular than in age-matched wildtype flies. This can clearly be seen by displaying the data in histogram format (see also Ocorr et al. 2007).

6. M-mode-

This module generates M-modes that can be made from any position along the heart in the movie frame. The length of the M-mode can also be specified. M-modes are useful to show qualitatively what the heart is doing in a movie.

Example (refer to Video): Movie of a 3 day old zebrafish larval heart showing the anterior ventricle that is used to generate an M-mode. The vertical slice of pixels that will be electronically excised from each movie frame is indicated by a red line.

M-mode Figure: Examples of M-modes from Drosophila and zebrafish. One of the myosin mutations discussed in the Preprocess step results in a molecular motor with faster enzyme kinetics resulting in heart restriction and occasionally a conduction block. Both parameters can be shown qualitatively using M-modes.



M-modes made in the same portion of the heart in both wildtype and mutant flies clearly show the restricted phenotype in the Mhc^5 mutant and the dilated diameters and arrhythmic contraction cycles of the D45 mutant (which expresses myosin with depressed enzyme kinetics) (modified from Cammarato et. al, 2008).

M-mode made from a Zebrafish larval heart showing the anterior ventricle (same heart movie as shown in the demonstration, Fink et.al. 2009).

7. Red Dot Movie-

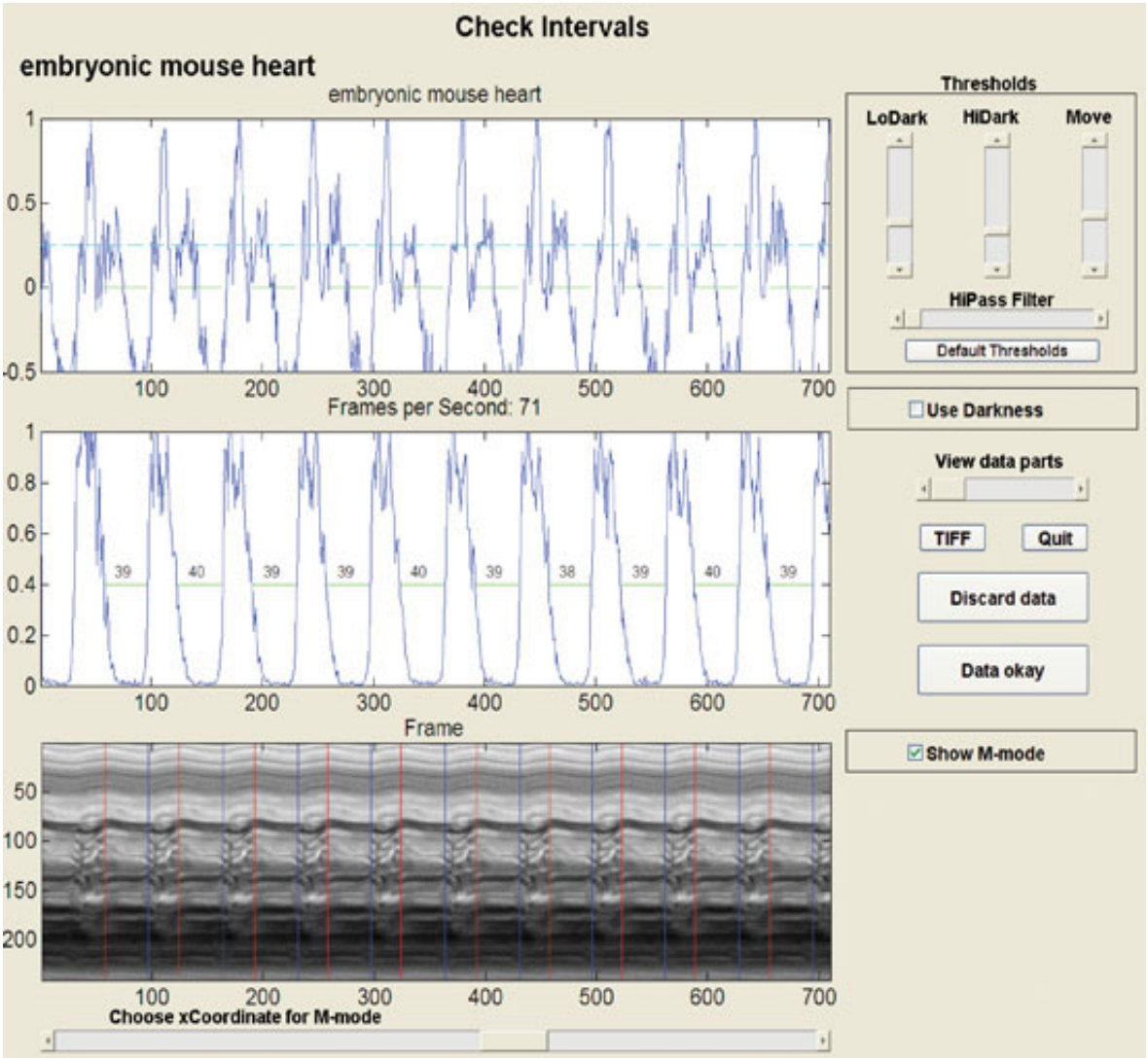
This module automatically produces a slowed (1:4), 20 second version of an analyzed movie showing all the pixels that are identified as changing in red. This feature is primarily used for illustration purposes.

Movie 1 - A "red dot movie" showing the pixels that are identified as having darkness intensity changes by the program algorithms in red. The movie speed is slowed from the original by a factor of 4.
Click here to download Movie 1.

8. Additional Applications-

We have begun to apply this analytical system to high speed movies of other models with small hearts which are often difficult to analyze using traditional methodology. We have successfully applied this analysis to both zebrafish larval hearts and embryonic mouse hearts (Fink et al, 2009).

Movie 2 - Mouse heart movie plus figure with check intervals with data output.
Click here to download Movie 2.



Discussion

The Drosophila model has proven to be a powerful genetic tool that has been used to address a variety of scientific questions ranging from embryological development to learning and memory. Recently this versatile model organism has been used to investigate the genetics of heart function. A number of attempts to quantify heart physiology in adult Drosophila have relied on observations made in intact flies through the abdominal cuticle. Most of these approaches depend upon visual observation or recordings of changes in light intensity transmitted through the abdomen to quantify a single parameter, heart rate. Although this is a useful parameter it is limited in what it tells us about heart function. Our Semi-automated Optical Heartbeat Analysis method is a robust approach for deriving accurate information about a number of additional important parameters from high speed movies of beating hearts and to do so for every heartbeat in a record.

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References

1. Cammarato A, Dambacher CM, Reedy MC, Knowles AF, Kronert WA, Bodmer R, Ocorr K, Bernstein SI. Myosin Transducer mutations differentially affect motor function, myofibril structure, and the performance of skeletal and cardiac muscles. *Mol Biol Cell* 19(2):553-62.(2008).
2. Ocorr K, Reeves N, Wessells RJ, Fink M, Chen H-SV, Akasaka T, Yasuda S, Metzger J, Giles W, Posakony JW, and Bodmer R. KCNQ potassium channel mutations cause cardiac arrhythmias in Drosophila that mimic the effects of aging. *Proc Natl Acad Sci U S A* 104:3943-8. (2007).
3. Fink M,Callol-Massot C, Chu A, Ruiz-Lozano P, Juan-Carlos Izpisua Belmonte, Giles W, Bodmer R, and **Ocorr K**. A new method for the detection and quantification of heartbeat parameters in Drosophila, zebrafish and embryonic mouse hearts. *Biotechniques* 46:101-113. (2009).

